Effects of Primary Sex Ratio on Operational Sex Ratio in Sea Urchin, *Echinometra mathaei*

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ABSTRACT

In the North of Persian Gulf region, sea urchins *Echinometra mathaei* are dominant in patches along the rocky coasts between the average low tide and a maximum depth of 5 m. In the current study, it was monitored monthly 217 individuals from Bandar Lengeh (26°32′28′N, 54°52′28′E; water depth: 0.5 to 1 m) during March to September, 2014. Their genders were obtained by dissection. The aim of this study was to determine the sexual ratio in their population and to see if they effect the operational sex ratio (OSR). The number of males and females were 71 and 126, respectively. Twenty samples by the total wet weights less than 12 g were non-sexual. The wet weight of the gonads was ~8.25% of the mean total wet body weights. The most distribution of gonad wet weights and total wet weights were generally less than 10 g and 80 g, respectively. The females were heavier than the males (~1.77x). The heavier females had more gonads (direct relation, r=0.45) while the lighter males had more gonads (inverse relation, r=-0.52). The males' total dry weights were < 20 g and > 40 g but females' were between 10 to 40 g. The total wet weight was > ~2x (females) and > ~7x (males) the total dry weight. The wet gonad weights is ~3x of the dry gonad weight of gonads and the two-thirds of total wet weight is the moisture of gonads. In females, Interquartile Range for gonad moisture content (GMC) was lower than the males or gonads of males had less moisture in compare with females. The operational sex ratio of *E. mathaei* was female-biased (OSR=0.36). The maximum and minimum of OSR was recorded in September (0.46± 0.11), and April (0.11± 0.11), respectively. It was not significantly different during the spawning period. The monthly sex ratios were significantly different during the spawning times. It was also observed an unstable condition for sex ratio and deviated from 1:1. The maximum of sex ratio was in April (1.07± 0.03) as the start of Spawning and September (0.86±0.02) as the recovery of gonad. Therefore, OSR cannot be affected by sex ratio at least at the start of Spawning time (April) and may be influenced by other parameters.

INTRODUCTION

An indicator used in animal communities that have separate genders is the operational sex ratio (hereafter, OSR). OSR was defined by Emlen and Oring (1977) as the proportion of sexually mature males divided by the total number of sexually mature adults at a given time and place. OSR is a major factor influencing the intensity of sexual selection (Clutton-Brock and Parker, 1992). The OSR may help elucidate sex differences during the life-history of a population (Kokko *et al.*, 2012). For example, in a population where 50% of males remain unmated, 50% mate once (Kokko *et al.*, 2012). Any change in OSR usually leads to increased competition for mates among all members of the more abundant sex, while members of the other sex may have a greater opportunity to exercise mate choice (Emlen and Oring, 1977). Sea urchins with separate

sexes and external fertilization, release their gametes into the sea at spawning time via five visible gonopores on their external shells. Since they have no special external sexual organs, the only way to recognize their genders can be distinguished after histological sectioning of the gonads (Pearse and Phillips, 1968).

Many species encounter spatial variation of OSR (Rohr *et al.*, 2005). In a male-biased situation, it is often correct to follow the traditional approach of this assumption that finding mates for females are easy (Kokko *et al.*, 2012) and females are selective (Balshine-Earn, 1996). A male-biased OSR can be affected by three major parameters: a) the adult sex ratio; b) the sex-specific time that individuals spend outside the mating pool; and c) environmental factors. The adult sex ratio is such as sex-biased mortality (Moore and Wilson, 2002), the primary sex ratio, and differential reproductive investment between the two sexes (Bateman, 1984). The sex-specific time that individuals spend outside the mating pool, are like providing parental care (Kokko and Jennions, 2008), replenishing gamete supplies, regaining body condition (Clutton-Brock and

Parker, 1992) or different mating behavior between sexes (Xiao and Kumar, 2004). Environmental factors are such as food supplies and hunger (Rowe *et al.*, 1994), nesting sites, predators (Gianguzza *et al.*, 2009), density of population (Gianguzza *et al.*, 2007) or even temperature which effects on breeding cycle (see Kvarnemo, 1994, as described previously).

Environmental factors can also effect the reproductive cycle of sea urchins, however, are not well understood. Pearse (1974) suggested that *Echinometra mathaei* might have a restricted spawning period in the higher latitudes and continuous spawning throughout the year closer to the equator where the environmental factors, especially temperature, are presumed more stable. It was found that different complicated factors effect spawning time of *Echinometra*, which were mainly environmental parameters as temperature, salinity, light, availability of food, the population density, wave-swept areas or even pH in different studies. The seasonal reproductive pattern of *E. mathaei* on the Kenyan coast is closely correlated to seawater temperatures, which are influenced by the monsoons (McClanahan, 1988). Interestingly, populations of *E. mathaei* at Rottnest Island, Western Australia, spawn continuously throughout the year in seawater temperatures that are cooler than on the Kenyan coast. Temperature variations may be the key factor in *E. mathaei* rather than an absolute temperature minimum or threshold for spawning. Carballeira *et al.* (2011) tested embryo-larval development (ELD) of sea urchin and its fertilization by a bioassays attempt to find out an optimum range of salinity (15 to 40.5 psu) with two species of Atlantic sea urchin: *Arbacia lixula* and *Paracentrotus lividus*. It was discovered that *A. lixula* (29 to 35.5 psu) had a wider salinity range rather than for *P. lividus* (29 to 33 psu). Lessios (1981) postulated that salinity could act as a proximate cue for controlling the timing of spawning as it coincided with increased salinity for *E. lucunter* and *E. viridis* at Fort Randolph, Caribbean. Light is another factor which effects spawning time (*e.g.,* Iliffe and Pearse, 1982). Temporal and spatial variability of reproductive conditions of sea urchins are closely related to trophic conditions (Scheibling and Hatcher, 2007). Fabbrocini and D'Adamo (2010) have tested the effects of food on gametogenesis of *Paracentrotus lividus* and found that starvation significantly affected gametogenesis, whereas developing gametes were always observed in fed animals. For example, growth and gonad production were lower in beds dominated by *Dictyopteris divaricata* and *Laurencia* spp. than in beds of small algae without defense chemicals (Agatsuma *et al.*, 2005). Moreover, the fertilization ability of gametes from starved urchins was significantly lower (Fabbrocini and D'Adamo, 2010). Even more, availability

of food for the larvae may be also an important factor in controlling spawning. Spawning occurs just prior to the peak of phytoplankton concentrations (Lessios, 1981). Lessios (1981) showed that spawning synchrony is tighter in sparsely populated populations of *E. viridis* and less important in densely populated populations of *E. lucunter*. (See also Muthiga and Jaccarini, 2005). Spawning also occurs in *E. lucunter* during a discrete period from July to October in wave-swept areas, and occurred during several periods in populations living in calm waters (Lewis and Storey, 1984). pH, (*e.g.,* Moulin *et al.*, 2011), the farm effluents, organic pollution (Cook and Kelly, 2007) and contamination of heavy metals (*e.g.,* Bayed *et al.*, 2005) can also effect spawning time.

Sex ratio is also important to the notion of echobiological and genetic balance for species in land and marine ecosystems. It provides information about the representations of sea urchin males and females (as the proportion of male to female), and indicates the gender dominance species in a given population and as mentioned can effect on OSR. As it is evident, the sex composition in any region is faced with extremely complex parameters and it can highly affects on the ratio of population.

The sex ratio of sea urchins especially *Echinometra mathaei*, is not previously studied on the north of Persian Gulf region, although they are the dominant, easily in patches along the rocky coasts between the average low tide and a maximum depth of 5 m. Therefore, the objective of the present paper was to study the OSR and its relationship with sex ratio of *E. mathaei* in the Bandar Lengeh area of the Persian Gulf.

MATERIALS AND METHODS

The study area was located in Bandar Lengeh, Iran, a small in*let al*ong a rocky shore in the northern part of the Persian Gulf (Fig. 1, which has provided by Arc GIS (ESRI 2011, Arc GIS Desktop, 64-bit, Version 10.3, Released 2011, Redlands, CA: Environmental Systems Research Institute.); station position: 26°32′28″ N, 54°52′28″ E; water depth: 0.5 to 1 m), covered with a high density of dominant macroalgae species of *Sargassum wightii* and *Padina antillarum*, from March to September, 2014. Due to the location of the studied area on subtropical zone, the first three months (March, April, May) had assumed as spring and the next four months (June, July, August, September) had considered as summer season.

Thirty one random size (height= [2.60 to 40.35 mm]) samples of urchins were collected individually each month using a handy stainless steel forceps in the intertidal zone during low tides of spring tides. They were transported in fiber tanks of sea water with continuous aeration by a
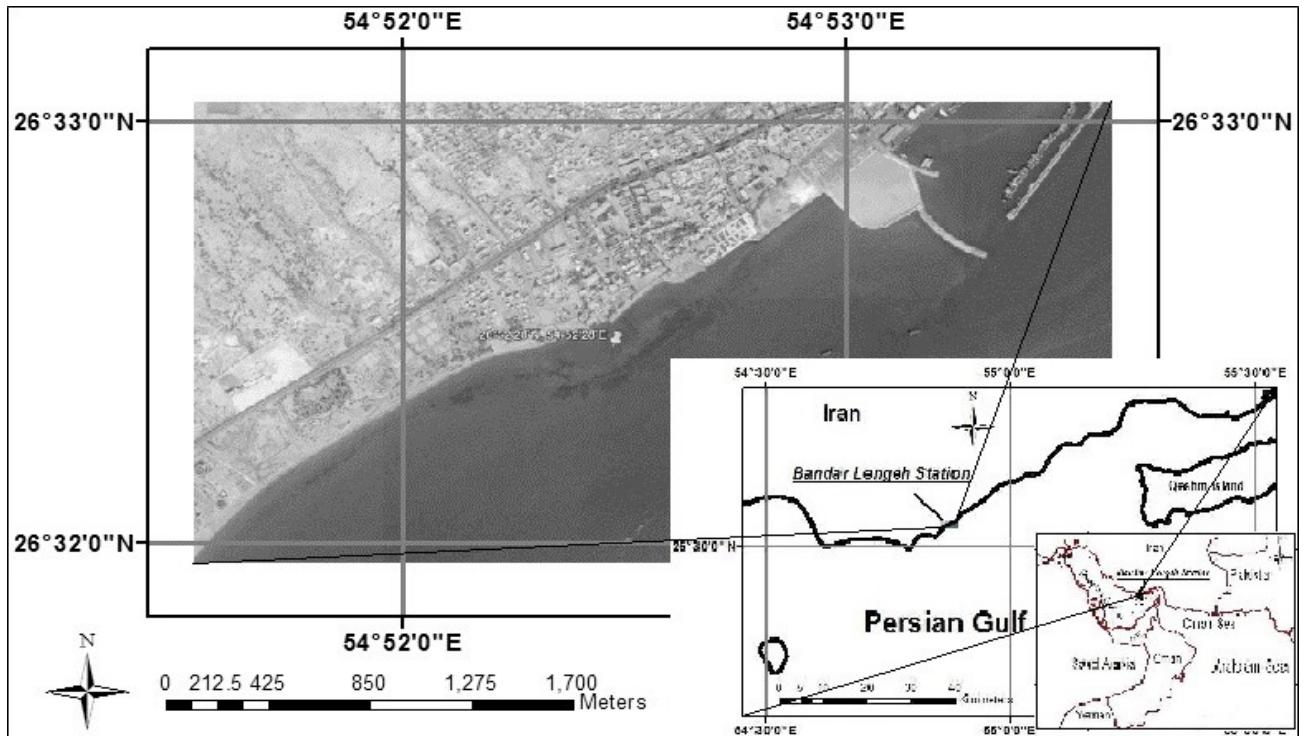
Fig. 1. A map of study area, Bandar Lengeh, in the Persian Gulf. Bottom left corner: The position of Persian Gulf; Bottom left middle: The position of Bandar Lengeh (map source: Iranian Army Geographic Agency); and finally the position of the station in Bandar Lengeh (map source: Google Earth). The rocky coasts are obvious in this map.

portable aquarium air pump (Model: Boyu D-200, Guangdong Boyu Group Company, Guangdong, China) of a 1.5V-waterproof battery as fresh *E. mathaei* to a marine biology laboratory in University of Hormozgan. It is useful to mention that the temperature of sea water during the total sampling months were 22.46 to 34.05 ˚C.

Before their dissections, every urchin was placed on a paper towel for about 1 to 2 min to eliminate any surface water, and the attached algae and crushed seashells of the urchins were discarded. The total wet weight of the animal was measured using a portable electric stainless steel balance [model: the professional digital table top scale (weighing scale 0.01 to 500 g, 0.01 g precision; Shenzhen Botoo Electronic Technology Company, Shenzhen, China)]. After measuring the weight, the dissection started. The spines of the urchins were removed by a slim pointed tip Lodestar stainless steel tweezers, L605014 (Limited Exempt Private Company, Singapore); the peristome was cut using a scalpel; the Aristotle's lantern was removed using forceps, and finally the coelomic fluid was removed. Two small cuts were made on the sample shell by using a scissors to divide the shell into two parts. The contents of the digestive system were removed and the urchin rinsed several times using sterile natural sea water. Then, the gonads were separated from the shell using small forceps, and kept inside pre-weighed 10 cc glass sterile vials. The wet weights of the gonads were obtained. And finally, sex was determined by observing a small piece of gonad using the easily observed color differences between males (light yellow-orange) and females (dark orange-brownish). OSR is defined as follows (Emlen and Oring, 1977):

$$OSR = \frac{Sexually\ mature\ males}{Total\ number\ of\ sexually\ mature\ adults} \dots \dots \dots (1)$$

The above formula was used to determine the monthly trend of male adults in compared with the general adult population and specify monthly differences. The primary sex ratio, hereafter sex ratio (males/females) was also calculated monthly.

$$Primary\ sex\ ratio = \frac{Males}{Females} \dots \dots \dots (2)$$

It was found useful to measure and analyze the weight of the gonads (Feng *et al.*, 2014) by equation 3, where "GMC" is gonad moisture content:

$$GMC = \frac{Wet\ Weight\ of\ the\ Gonad - Dried\ Weight\ of\ the\ Gonad}{Wet\ Weight\ of\ the\ Gonad} \times 100 \dots \dots \dots (3)$$

It is useful to mention that for achieving dry weights of each individual on equation 4, samples were put inside a 60 ˚C oven for 24 hours, and then they were weighted as "primary dry weights". This step has repeated continuously until a fixed unchanged weight named "Dry Weight" was found for each individual sample in compare with previous step.

Statistical analysis

Statistical analysis used different software programs suitable for Windows: IBM SPSS (IBM Corp. 2013, IBM SPSS Statistics for Windows, 32-bit, Version 22.0.0.0, Released 2013, Armonk, New York.); MINITAB (Minitab Inc. 1996, MINITAB statistical software, 32-bit, Version 11.12; Minitab Release 11, State College, Pennsylvania, USA), and Microsoft office Excel (JKP Application Development Services, 2007, Microsoft Office Excel, 32-bit, Version 2007, Weert, The Netherlands) hereinafter called SPSS, MINITAB, and Excel respectively.

The gonad weights and total wet weights distributions of *E. mathaei* over the whole sampling period (March–September, 2014) were calculated by using SPSS (Mean ± Standard Deviation). The data outputs were also checked by Skewness to see whether the data was symmetric (-2≤Standard Error of Skewness≤2) or asymmetric (-2> Standard Error of Skewness>2). The most frequent data of each parameter were obtained by calculating frequency and plotting their distributions using SPSS.

The data checked for the homogeneity of the variance and normal distribution in SPSS. OSR (equation 1), sex ratio (equation 2) were compared statistically by one-way ANOVA. Chi-squared test was used to see if the monthly proportion of males and females deviated from 1:1 (at the statistical significant level $P < 0.05$). Significant differences of OSR among months were tested by Tukey's HSD (at the statistical significant level $P < 0.05$) by Excel. Excel graphs were also prepared the OSR.

Finally, MINITAB was also used to calculate the gonad moisture content index (GMC) for the two genders. It was also used to calculated Quartiles ($Q_1(x_i)$ and $Q_3(x_i)$), Interquartile Range ($Q_3(x_i) - Q_1(x_i)$), and Range (Maximum (x_i) – Minimum (x_i)), where x_i refer to males, females, or total population for equation 3.

RESULTS AND DISCUSSION

During the whole sampling period (March to September, 2014), 217 individuals of *E. mathaei*, from Persian Gulf, Bandar Lengeh, were studied. There were 71 males, 126 females while the gender of 20 samples was not obvious because they were too small without any gonad. The largest sample was by the weight of 103.77 g while

the smallest was 0.07 g. The numbers of female, male and unsexual samples were 59, 31 and 7 in spring and 67, 40 and 13 in summer, respectively.

Gonad weights compared to the total weight of the body is of great importance because of their economical value. Figure 2 presents the distribution of the measured weights for all samples by genders. In Bandar Lengeh, according to Figure 2A, defining the gender of samples by total weights less than 12 g, was mostly difficult because those samples had no gonad. The highest frequency for the total weights was 40 to 44 g (both females and males). The frequency of total weights of females was more than males. It means that females were heavier (<92 g) than males (<70 g) in the studied samples. Figure 2B also shows the gonad weight was less than 16 g, with most being less than 10 g.
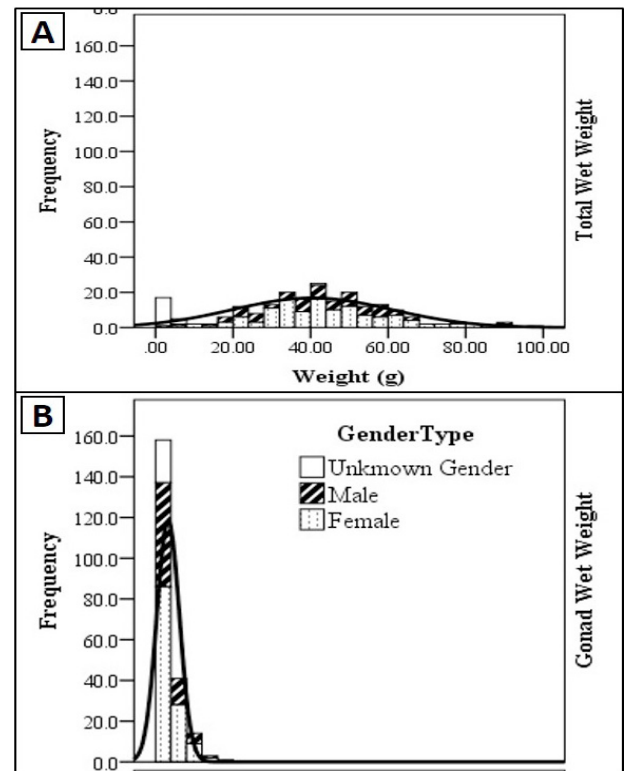

Fig. 2. Distributions of weights of 217 *E. mathaei* samples (March to September, 2014) by gender. **A,** Total Wet Weight (40.38±20.59 g); **B,** Wet Weight of Gonads (3.13±2.72 g).

Table I represents the most frequent data of each parameter and how symmetric they are based on Skewness. Multiple high frequencies were seen for wet weights of gonads. It shows obviously that the mean wet weight of gonads was nearly 8.25% of the mean total body weight. The most frequent wet weights of gonads (female: 80,

male: 60, unknown sex: 20) was less than 4 g. A sharp drop in frequency was observed for gonad wet weights of 5 to 12 g. Only females had in gonad wet weights of 12 to 16 g. Figure 2 suggests that the total wet weight distribution was wider than gonad weight distribution. Briefly, the gonad wet weight distribution was not normally distributed.

Table I.- Sea urchin weight and gonad weight, derived by SPSS analysis.

Parameter	Weight Of Wet Gonads (g)	Total Weight (g)
Mean ±SD	3.30 ± 2	40.00 ± 21
Skewness	1.40	0.08
Symmetric/asymmetric	Asymmetric	Symmetric
Most frequent data	0.00–0.83, 2.51–3.34	35.0–45.0

The monthly variation of weights is also represents (Fig. 3). Total wet weights were between 31.8 g (in August) and 48.7 (in July), while the dry weight range was 15.6 g (August) to 22.24 g (September). A slight decrease is obvious on the gonad wet weight in March (6.13 g) to August (1.08 g) the same as the gonad dry weight (1.50 in March to 0.41 in August). It is clear that March and April are the start of spawning time by higher values and August is the end of spawning time by lower values. Recovery of gonads start in September.
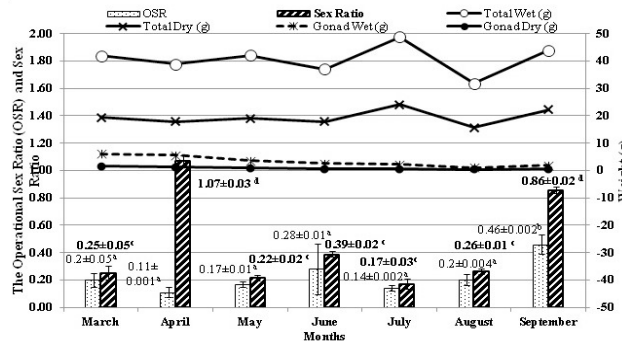

Fig. 3. Different parameters of *E.mathaei* from March to September, 2014. Bars (left vertical axis): the operational sex ratio and sex ratio (Mean ± Standard Deviation). The different alphabet above numbers as a superscript show significant differences between months (p<0.05). Lines (right vertical axis): the total wet weights, the total dry weights; the gonad wet weights, and the gonad dry weights.

On Figure 4 and 5, scatter plots of weights are provided due to the gender by Excel. As the Figure 4 illustrates weights of samples mostly scattered for total wet weight < 80 g and total dry weight < 40 g. The non-

sexual samples often had total dry weights < 5 g and total wet weight < 20 g. For each sample total wet weight was almost more than double total dry weight (the slope of the linear estimated equation it 0.43) in females, while total dry weight was seven times more total wet weight in males (the slope=0.13). The correlation coefficient of total wet weights and total dry weights in females, males and non-sexual samples were 0.92, 0.23 and -0.20, respectively.
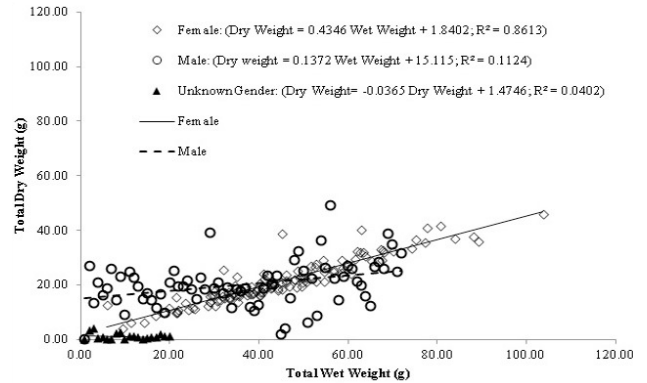

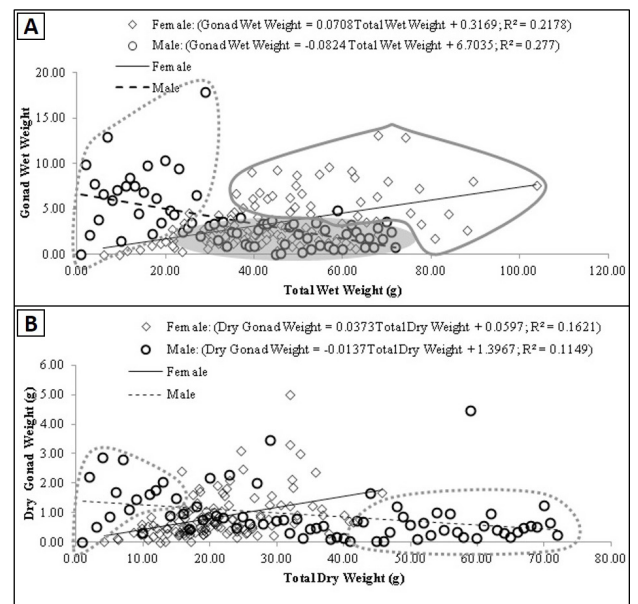Fig. 4. The relationship between total wet and dry weights.

Fig. 5. The relationship between gonad and total weights. **A,** wet weights (females, gray solid line; males, gray dotted line; the two sex combinations: gray shaded zone); **B,** dry weights (females, gray solid line; males, gray dotted lines).

The most distribution of gonad wet weights and total wet weights were generally less than 10 g and 80 g, respectively (Fig. 5A). If was found three special zones

(Fig. 5A): higher total wet weights are females (gray solid line); lower values are only males (gray dotted line) and their combinations (gray shaded zone). The direct relationship of gonad wet weight and total wet weight was found for females (r=0.45) and the inverse one for males (r=-0.52). In the other words, the gonad wet weights of females are more than males. Samples total wet weighing less than 70 g usually had gonad dry weight less than 2 g (Fig. 5B). The males' total dry weights were < 20 g and > 40 g but females' were between 10 to 40 g. As a result of the comparison of Figure 5A and B that the wet gonads weights is ~3x of the dry gonad weight of gonads and the two-thirds of total wet weight is the moisture of gonads.

The GMC (equation 3) using MINITAB is shown in Table II according to sex and total samples. It is found out the mean of GMC has no significant differences between populations. In females, Interquartile Range for GMC was lower than the males. Since GMC is the ratio of the difference between wet and dry weight to wet weight of gonads, and Range is the difference between maximum and minimum of GMC, then it was found the lower values of GMC in males than females. It means that gonads of males had less moisture in compare with females.

Table II.- The gonad moisture contents index (GMC) for the two genders of *E. mathaei*.

Parameters	No.	Mean ± SD	FQ	TQ	IR	Range
Male	71	74 ± 10	70	81	11	57
Female	126	75 ± 11	72	80	7.6	91
Total	217	68 ± 24	70	80	10	99

FQ, First Quartile; TQ, Third Quartile; IR, Interquartile Range.

By using equation 1, the monthly OSR was calculated for *E. mathaei* and presented on Figure 3. The maximum and minimum of OSR (Mean± Standard Deviation) was recorded in September (0.46± 0.11), and April (0.11± 0.11), respectively. OSR was significantly different between months (P<0.05). Moreover, the maximum and minimum of monthly sex ratio (Mean± Standard Deviation) was 1.07± 0.03 (April) and 0.17± 0.04 (July), respectively. Since spawning start in April, sex ratio is nearly 1:1; but during spawning, sex ratio decreases till September when recovery of gonads starts (See Fig. 3) The September and April sex ratio were significantly different with other months (P<0.05). There was a significant deviation from an overall sex ratio of 1:1 (χ^2=0.05, df=1, p=0.157) without any hermaphrodites samples.

In this study, the sex ratio of *E. mathaei*, showed no significant differences in individual males and females by the Chi-square test (p=0.157 (P>0.05)). It means that there is no preference between males and females as the primary sex ratio and then no effects on OSR. No significant differences were also found by Bronstein and Loya (2014), from a ratio of 1:1 for *Echinometra* sp. in Zanzibar. Zhao *et al*. (2010) also found no significant differences by the Chi-square test for *Strongylocentrotus intermedius* in China.

In the study of *E. mathaei*, we have found that the sex ratios, had significant differences between months (April and September with other months) by ANOVA-test, (P<0.05) but OSR had no significant differences between months except September (by ANOVA-test, P>0.05). Gianguzza *et al*. (2009) showed that the OSR had no significant differences (P>0.05 and P>0.001) for *Paracentrotus lividus*.

As it is described on the introduction, sex ratio and OSR depend on many parameters and face with complex situation. Since sea urchins sampling was random, it cannot be exactly stated that what parameters affects the sex ratio. Among all those described parameters, the parental cares cannot certainly effects the sex-specific time that sea urchins spend outside the mating pool. Others like replenishing gamete supplies, regaining body condition or different mating behavior between sexes might be important. Environmental factors such as food supplies and hunger, nesting sites, predators, density of population or even temperature which effects on breeding cycle can also effects of OSR as described previously. As an example, the females of sea urchin *Paracentrotus lividus* were more vulnerable to the starfish *Marthasteria glacialispredation* at high densities of this starfish during the summer spawning period and therefore OSR is female-biased (Gianguzza *et al*., 2009). Or another example, if males are required to provide a nutrient high gift before mating (most likely food) then when the available nutrients are high, the OSR will be male biased because there are plenty of nutrients available to provide gifts. However, if nutrients is low, less males will be ready to reproduce, causing the population to have a female biased OSR. In this study of *E. mathaei*, since the standard definition of OSR is adult males to total adults (equation 1), it is assumed that OSR should be male-biased, but surprisingly it was found that OSR in this species is female-biased (male: 71; female: 126). It means that adult females are roughly 1.77 times in compare with adult males. In another study on *Paracentrotus lividus*, it was suggested that OSR depends on density, so that, at higher densities, there was a more equal sex ratio whereas at low density sites males of *P.lividus* were significantly more abundant (Gianguzza *et al*., 2007) and therefore OSR is male-biased. Another male-biased OSR for *Strongylocentrotus franciscanus* was also found at intermediate densities by the study of Levitan (2004). Levitan (2004) mentioned that at low and

high densities, OSR is roughly 50% of total for both sexes. It means that our study for *E. mathaei*, in Bandar Lengeh is completely opposite with those two studies of *P.lividus* and *S. franciscanus*. But on the other hand, by regarding to ANOVA-test, we had the same results as Gianguzza *et al.,* (2009). Overall, due to no effects of sex ratio on OSR, more studies are required to check the effects of time spending outside mating pool and spatially environmental factors on OSR to understand why *E. mathaei* is female-biased in Bandar Lengeh, Iran.

CONCLUSIONS

In this study, we have collected random size of *E. mathaei* once per month (March – September, 2014), Bandar Lengeh, Iran. Their gonads were tested by considering Operational Sex Ratio (OSR). We have discovered out *E. mathaei* is female-biased and consequently finding mates for males is convenient. It was also found out March to August, 2014, as spawning months of this species. Since sex ratio has no effects on OSR, therefore, the other factors can affect OSR: 1) the time that individuals spend outside the mating pool; 2) regaining body condition; 3) different mating behavior between sexes; 4) environmental factors, which all are needed continuous studies during years on *E. mathaei* natural conditions of Bandar Lengrh; and finally, 5) different replenishing gamete supplies of females and males that can be more highlighted because of a female-biased OSR in *E. mathaei*.

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Conflict of interest statement
We declare that we have no conflict of interest.

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